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Combining the α_1-Adrenergic Receptor Antagonist, Prazosin, with the β-Adrenergic Receptor Antagonist, Propranolol, Reduces Alcohol Drinking More Effectively Than Either Drug Alone

Dennis D Rasmussen, PhD[1,2,3], **Lauren E Beckwith, BS**[2,3], **Carrie L Kincaid, BA**[2,3], and **Janice C Froehlich, PhD**[4]

Dennis D Rasmussen: drasmuss@u.washington.edu

[1]VISN 20 Mental Illness Research, Education and Clinical Center

[2]VA Puget Sound Health Care System, Seattle, WA 98108

[3]Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA 98195

[4]Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202-5124

Abstract

Background—Evidence suggests that activation of the noradrenergic system may contribute to alcohol drinking in animals and humans. Our previous studies demonstrated that blocking α_1-adrenergic receptors with the antagonist, prazosin, decreased alcohol drinking in rats under various conditions. Since noradrenergic activation is also regulated by β-adrenergic receptors, we now examine the effects of the β-adrenergic receptor antagonist, propranolol, alone or in combination with prazosin, on alcohol drinking in rats selectively bred for high voluntary alcohol intake and alcohol preference (P line).

Methods—Two studies were conducted with male P rats. In study one, rats were allowed to become alcohol-dependent during 14 weeks of ad libitum access to food, water and 20% alcohol and the effect of propranolol (5–15 mg/kg, IP) and prazosin (1–2 mg/kg, IP) on alcohol intake during withdrawal were assessed. In study two, the effect of propranolol (5 mg/kg, IP) and prazosin (2 mg/kg, IP) on alcohol intake following prolonged imposed abstinence was assessed.

Results—Alcohol drinking following propranolol treatment was variable, but the combination of propranolol + prazosin consistently suppressed alcohol drinking during both alcohol withdrawal and following prolonged imposed abstinence, and the combination of these two drugs was more effective than was treatment with either drug alone.

Conclusions—Treatment with prazosin + propranolol, or a combination of other centrally active α_1- and β-adrenergic receptor antagonists, may assist in preventing alcohol relapse in some individuals.

Correspondence: Dennis D Rasmussen, PhD, 116 MIRECC, VA Puget Sound Health Care System Medical Center, 1660 S Columbian Way, Seattle, WA 98108.

Keywords

propranolol; prazosin; alcoholism treatment; noradrenergic

INTRODUCTION

Although alcoholism remains the most prevalent addictive disease, effective treatments are few. Psychosocial therapies are associated with relapse rates of 40–70% in the first year (Finney et al., 1996). Current US Food and Drug Administration (FDA) approved pharmacologic treatment options – disulfiram, acamprosate, and naltrexone – primarily interact with aldehyde dehydrogenase, glutamatergic systems, and opioidergic systems, respectively, and all have variable limitations to their use and effectiveness (Garbutt, 2009). Additional treatment targets and strategies are needed.

Noradrenergic activation in the central nervous system (CNS) plays an important role in regulation of alcohol drinking [for recent discussions, see (Rasmussen et al., 2009a) and (O'Neil et al., 2013)]. Evidence for noradrenergic regulation of alcohol drinking comes largely from recent studies with prazosin, a specific α_1-adrenergic receptor antagonist that is non-subtype selective (similar affinities for α_{1A}, α_{1B}, α_{1D}). Prazosin blocks CNS α_1-adrenergic receptor-mediated signaling when administered systemically at clinically relevant doses (Menkes et al., 1981; Rojawski and Aghajanian, 1982). Prazosin treatment decreases alcohol withdrawal-induced operant alcohol intake in Wistar rats (Walker et al., 2008); decreases voluntary alcohol drinking by selectively-bred alcohol-preferring (P) rats (Rasmussen et al., 2009a); facilitates abstinence by treatment-seeking alcoholics (Simpson et al., 2009); increases latency to first operant response for alcohol by P rats (suggesting decreased motivation to initiate alcohol consumption) (Verplaetse et al., 2012); reduces alcohol drinking throughout prolonged treatment of P rats (Froehlich et al., 2013a); blocks initiation of alcohol drinking by P rats (Froehlich et al., 2013a); blocks development of alcohol conditioned place preference in mice when microinjected into the brain ventral tegmental area (Raskind et al., 2011); decreases stress-induced reinstatement of alcohol seeking in rats (Lê et al., 2011); and decreases stress- and cue-induced alcohol craving in alcohol-dependent men and women (Fox et al., 2012). This growing body of literature demonstrates that reducing α_1-adrenergic receptor-mediated activity in the CNS reduces alcohol drinking and relapse in rodents and humans, and provides a new target for potentially effective pharmacotherapy for alcohol abuse disorders.

Central noradrenergic activation is mediated not only by α_1- but also by β-adrenergic receptors (Cooper et al., 2003; Westfall and Westfall, 2006). We now investigate the potential role of β-adrenergic receptors in mediating alcohol drinking by using propranolol, a non-subtype-selective (equal affinity for β_1 and β_2) antagonist of β-adrenergic receptors that, like prazosin, is CNS active when administered systemically (Westfall and Westfall, 2006). The results of prior studies on the effects of propranolol on alcohol drinking are conflicting. Propranolol did not alter 24-hour free-choice alcohol drinking in Long Evans rats (Begleiter, 1974), but did decrease alcohol preference in mice (Andreas et al., 1983). Alcohol withdrawal symptoms that are blocked by prazosin in alcohol-dependent rats are

likewise blocked by propranolol (Trzaskowska and Kostowski, 1983), and propranolol decreased alcohol withdrawal-induced acute operant responding for alcohol in rats made alcohol-dependent by inhalation of alcohol vapor (Gilpin and Koob, 2010). However, an effect of propranolol on operant self-administration of alcohol has been reported to be dependent upon history of alcohol dependence (Gilpin and Koob, 2010). The goal of the current investigation was to further evaluate the utility of α_1- and β-adrenergic receptor antagonists as potential agents for treatment of alcohol use disorders, by: 1) exploring potential β-adrenergic regulation of alcohol drinking by P rats using a protocol that we previously used to demonstrate a role for the α_1-adrenergic system in alcohol drinking by P rats (Rasmussen et al., 2009a; O'Neil et al., 2013; Froehlich et al., 2013a, 2013b; Rasmussen et al., 2009b); and 2) assessing potential interactions between α_1- and β-adrenergic regulation of alcohol drinking. In a preliminary investigation (Rasmussen et al., 2011), effects of propranolol on alcohol drinking by male P rats were inconsistent and confounded by hypnotic–like responses to high doses of propranolol. Consequently, in the present study we investigated effects of moderate doses of an α_1- and a β-adrenergic receptor antagonist, alone or in combination, on alcohol intake in alcohol-dependent P rats during withdrawal and following prolonged imposed abstinence, with testing to identify potentially confounding hypnotic drug effects if present.

METHODS

Subjects

Alcohol-naïve male rats from generation 70 of selective breeding for alcohol preference were provided by the Alcohol Research Resource Center of the Indiana Alcohol Research Center. These alcohol-preferring (P) rats were 50 ± 1 days of age and 237 ± 3 g at the start of the study (n = 46). All rats were individually housed in stainless steel hanging cages in an isolated vivarium with controlled temperature (21 ± 1°C) and reverse 12-hour light/dark cycle (lights off at 1000 hour; procedures during the dark period were performed under dim red illumination). All rats were acclimated to individual housing and the light/dark cycle for 10 days before the study. Chow (Laboratory Rodent Diet 5001, PMI Nutrition International, Brentwood, MO) and water were available *ad libitum* except during the first 3 days of the alcohol drinking induction phase when 10% (v/v) alcohol was the only source of fluid. All experiments were approved by the Veterans Administration Puget Sound Health Care System Institutional Animal Care and Use Committee and conducted in compliance with the NIH Guide for the Care and Use of Laboratory Animals.

Alcohol and Water Intake

Alcohol solutions were prepared by diluting 95% alcohol (ethanol) with deionized water. Alcohol and water were presented in two adjacent glass drinking tubes (BioServ, Frenchtown, NJ) with positions alternated daily to control for potential side preferences. Fluid intakes were determined by weighing each tube to the nearest 0.1 g. Alcohol and water tubes were also placed on two empty cages to determine loss due to spillage and/or evaporation; average losses in these two cages on each day were subtracted from intake for that day. Net alcohol intake was converted to g alcohol/kg body weight.

Alcohol Drinking Induction Phase

Before providing prolonged ad libitum access to alcohol in order to produce dependence, 2 hour/day alcohol drinking was established to familiarize the rats with the reinforcing properties of alcohol within a limited-access paradigm (i.e., all rats were given 10% [v/v] alcohol as the only source of fluid ad libitum for 3 days, followed by 24-hour/day free-choice between water and 10% [v/v] alcohol for 10 days; access to alcohol was then reduced to 2 hours/day [1100 – 1300 hours] for 5 days/week and after 2 weeks the alcohol concentration was increased to 15% [v/v] for 2 weeks). Alcohol and water intakes during each 2-hour alcohol access period were recorded daily and body weight was recorded weekly.

Drugs

Propranolol hydrochloride and prazosin hydrochloride (both from Sigma-Aldrich, St. Louis, MO) were dissolved in 45 mM lactate buffer (pH 5.2). Propranolol, prazosin, [propranolol + prazosin] or lactate buffer vehicle were injected intraperitoneally (IP; 4 ml/kg body weight) 30–40 minutes before the daily 2-hour alcohol access period. The plasma half-life of prazosin in the rat has not been determined but is approximately 3 hours in the human (Westfall and Westfall, 2006); the plasma half-life of propranolol in the rat is 1–1.5 hours (Belpaire et al., 1990).

STUDY 1: effects of propranolol alone or propranolol + prazosin on alcohol intake during withdrawal—After 2-hour/day scheduled-access alcohol drinking had been established in the alcohol drinking induction phase, 38 of the rats were given ad libitium access to 20% (v/v) alcohol for 14 weeks interrupted by 2 consecutive days of alcohol deprivation during each of the final 5 weeks; the remaining 8 rats did not receive further alcohol during this 14 week period. Alcohol access was terminated after 14 weeks and, 9 hours later, behavioral signs of alcohol withdrawal were evaluated in all rats (i.e., those with and without 14 weeks of ad libitum alcohol access). Withdrawal was characterized using the protocol developed by Macey et al. (1996) as previously described (Rasmussen et al., 2001). Briefly, each rat was rated on three behaviors (abnormal body posture; tail stiffness; and ventromedial distal limb flexion response) using scales of 0 to 2 for each behavior. The three scores were summed for a withdrawal rating of 0 to 6 for each rat; 0 corresponds to undetectable and 6 corresponds to severe withdrawal. Testing of the rats that had received 14 weeks of 20% alcohol access *vs* rats that had not received ad libitum access to 20% alcohol was conducted in randomized order by two investigators blinded to treatments.

At completion of withdrawal testing, the 8 rats that had not received 14 weeks of ad libitum access to 20% alcohol (withdrawal controls) were removed from the study. The remaining 38 rats were again given ad libitum access to 20% alcohol before the effects of propranolol or prazosin on alcohol intake were assessed in a series of three experiments (1a, 1b, 1c in the timeline presented in Fig. 1). Each experiment was preceded by 2 weeks ad libitum access to 20% alcohol, with average alcohol intake during the second week used to counterbalance and assign rats to one of 4 treatment groups (9–10 rats/group) in a manner which assured that the groups did not differ in baseline alcohol intake prior to drug administration, as

previously described (Rasmussen et al., 2009a). Each experiment was initiated by shifting the rats from 24-h alcohol access to daily 2-hour alcohol access (1100 – 1300 hour free-choice between 20% alcohol and water). The first daily 2-hour alcohol access was provided at 5 hours after termination of the 24-hour alcohol access; daily 2-hour alcohol access sessions were provided on each of 4–6 subsequent days, with drugs administered IP 30–40 minutes prior to each daily alcohol access. At the completion of each experiment, all rats were again given 24 hour alcohol access for 2 weeks prior to the start of the next experiment. In experiment 1a, the treatment groups received propranolol (5, 10 or 15 mg/kg) or vehicle. In experiment 1b, the groups received propranolol (10 mg/kg), prazosin (1 mg/kg), prazosin (1 mg/kg) + propranolol (10 mg/kg), or vehicle alone. In experiment 1c, the groups received propranolol (5 mg/kg), prazosin (1 mg/kg), prazosin (1 mg/kg) + propranolol (5 mg/kg), or vehicle alone on the first 3 days; on day 4 the dose of prazosin in both the prazosin and the [prazosin + propranolol] treatment groups was increased to 1.5 mg/kg, and on day 5 it was further increased to 2 mg/kg. On day 5 the effect of prior drug treatment on arousal and ingestive behavior were also tested. In the first test, each rat was observed at the onset of 2-hour alcohol access to determine if the rat investigated (sniffed, licked) and/or drank from the tubes within 1 minute (which would indicate that the rat was not sleeping or sedated and was motivated and competent to investigate the tubes). The second test was conducted at completion of the 2-hour alcohol access period when the drinking tubes were removed; a chow pellet (identical to pellets available in the food hopper) was slipped into the cage through the hole that had accommodated the alcohol tube to determine whether the rat chewed on the pellet within 15 seconds (which would indicate that the rat was not sleeping or sedated at the end of the 2-hour alcohol access period).

STUDY 2: effects of propranolol and/or prazosin on alcohol intake following prolonged imposed abstinence—After completion of the three experiments in Study 1, the rats were deprived of alcohol for 3 weeks, as illustrated in the timeline in Fig. 1. Alcohol and water intakes were then determined in a single 2-hour 2-bottle choice (20% alcohol *vs* water) session starting 1 hour after lights-off, without drug treatments. The rats were again ranked in order of alcohol intake in this single 2-hour access and re-assigned as previously described to treatments for 5 consecutive days in Experiment 2. On each of days 1 and 2 the rats were given 2-hour 2-bottle choice between 20% alcohol and water with propranolol (5 mg/kg), prazosin (2 mg/kg), prazosin (2 mg/kg) + propranolol (5 mg/kg), or vehicle alone administered 30–40 minutes prior to alcohol access. On day 3, the drug treatments were again administered at 20–30 minutes after lights-off but no alcohol access was provided; this allowed for testing of drug effects on locomotor activity and ability to drink, independent of concurrent pharmacologic effects of alcohol. At 40 minutes after drug injections (i.e., when alcohol access would normally begin), 1 ml of a sweet solution (2% sucrose + 0.2% saccharin) was presented to each rat in a cup secured to prevent tipping (the rats each had received 1 ml of this sweet solution on 3 previous occasions to discover its palatability and minimize neophobia). It was determined whether each rat consumed the entire 1 ml of sweet solution within 1 minute. This test was repeated at 100 minutes after the drug injections, i.e., at the midpoint of the 2-hour period. Between the two tests of sweet solution consumption, each rat was transferred to a separate cage in the same room for a 5 minute measurement of locomotor activity, assessed as the number of infra-red beam breaks using an Opto-Varimex

Mini monitoring system (Columbus Instruments, Columbus, OH). On day 4, drugs were administered as on days 1 and 2 at 30–40 minutes prior to the onset of 2-hour alcohol access and the combined effects of drug administration + alcohol drinking on ingestive behavior was assessed at the midpoint of the 2-hour alcohol access with another presentation of 1 ml of the sweet solution, followed by determination of whether the rat consumed all of the solution within 1 minute. On day 5, drug treatment prior to alcohol access continued but alcohol access was terminated after 1 hour and tail blood was collected for determination of alcohol concentration by NAD-ADH enzymatic Ethanol Assay (Genzyme Diagnostics; Charlottetown, PE, Canada).

Data Analyses

The Grubbs' Test (Extreme Studentized Deviate Method) performed with GraphPad QuickCalcs (GraphPad Software, San Diego CA USA, www.graphpad.com) was used to eliminate outlying ($p<0.05$) alcohol or water intake values that were likely due to leakage or spillage. No more than one intake determination in any [treatment X day] group was designated as an extreme outlier.

Alcohol and water intakes during drug treatments were analyzed by two-way (dose X day) analyses of variance (ANOVA) with repeated measures on day, followed – when justified by significant ANOVA main effects or interactions – by pairwise comparisons using Student-Newman-Keuls (SNK) tests, unless otherwise noted. Two-way ANOVA main effect statistics are reported only when there was not a significant interaction requiring individual dose X day comparisons. Pre-and post-treatment alcohol and water intakes, locomotor activities, and plasma alcohol levels within an individual day were each analyzed by one-way ANOVA followed – when justified by a significant difference in the ANOVA – by pairwise SNK comparisons. Associations between blood alcohol concentration (BAC) and preceding alcohol intake were evaluated by Pearson Product Moment Correlation analyses. Analyses were conducted using Sigmaplot Version 11 software (Systat Software, Inc., Chicago,IL) with significance accepted at $p < 0.05$. Data are presented as mean ± SEM.

RESULTS

STUDY 1, alcohol dependence

Rats that received 24-hour/day access to 20% alcohol for 14 weeks exhibited increased alcohol withdrawal behavior scores relative to control rats (2.9 ± 0.2 and 1.4 ± 0.5, respectively; $p < 0.01$ by Student t-test).

STUDY 1, Experiment 1a—The effects of propranolol treatment on 2-hour alcohol drinking in alcohol-dependent P rats during withdrawal from prolonged ad libitum alcohol drinking are presented in Fig. 2. There were significant effects of dose, $F(3, 34) = 4.38$, $p = 0.01$, and day, $F(4, 122) = 6.59$, $p < 0.001$, but no significant dose X day interaction during the five drug treatment days. Alcohol intake was suppressed by propranolol in doses of 10 or 15 mg/kg (each $p < 0.05$, relative to vehicle control), independent of day. With respect to water intake (data not shown), there was a significant dose X day interaction, $F(12, 109) =$

2.04, p <0.05. Only the highest dose of propranolol (15 mg/kg) decreased water intake, and only on drug day 4 ($p < 0.05$).

STUDY 1, Experiment 1b—The effects of propranolol 10 mg/kg, prazosin 1 mg/kg, or the combination of [propranolol 10 mg/kg + prazosin 1 mg/kg] on 2-hour alcohol intake during the first 4 days of withdrawal from 24-hour/day alcohol access are presented in Fig. 3. There was a significant treatment × day interaction, $F(9, 74) = 2.60$, $p < 0.05$; propranolol treatment increased alcohol intake on day 3 ($p < 0.01$) and [propranolol + prazosin] decreased alcohol intake on days 1, 2, and 3 ($p < 0.01$ for each) relative to treatment with vehicle alone. With respect to water intake (data not shown), there was a significant treatment X day interaction, $F(9, 77) = 2.03$, $p < 0.05$; water intake on day 4 was greater in rats receiving [propranolol + prazosin] than in rats receiving propranolol alone ($p < 0.05$), but there were no effects of drug treatment relative to vehicle treatment. There were also no significant effects of previous drug treatments on either alcohol or water intakes on the post-drug day.

Alcohol preference (ml of alcohol / [ml of alcohol + ml of water]) was similarly analyzed. There was a significant effect of drug treatment, $F(3, 33) = 4.33$, $p \leq 0.01$, and a significant effect of treatment day, $F(3, 9) = 14.86$, $p < 0.001$, but no significant treatment X day interaction. Treatment with [propranolol + prazosin] decreased alcohol preference compared to treatment with vehicle or treatment with propranolol alone ($p < 0.05$ for each), independent of day. The average 4-day alcohol preference was 0.76 ± 0.04 for vehicle, 0.78 ± 0.05 for propranolol, 0.67 ± 0.05 for prazosin and 0.56 ± 0.04 for [prazosin + propranolol]. There was no significant effect of previous drug treatment on the post-drug day alcohol preference.

STUDY 1, Experiment 1c—The effects of propranolol and/or progressively increasing doses of prazosin on 2-hour alcohol intake during five days withdrawal from 24-h/day alcohol access are shown in Fig. 4. On days 1–3 (when each rat received either vehicle, propranolol 5 mg/kg, prazosin 1 mg/kg, or [prazosin 1 mg/kg + propranolol 5 mg/kg] on each day) there was a significant effect of drug treatment, $F(3, 32) = 10.35$, $p < 0.001$, but no effect of day and no treatment × day interaction. Treatment with [prazosin 1 mg/kg + propranolol 5 mg/kg] decreased alcohol intake independent of day, relative to treatment with vehicle, prazosin 1 mg/kg, or propranolol 5 mg/kg ($p < 0.001$ for each). On day 4, when the prazosin dose was increased to 1.5 mg/kg (i.e., each rat then received either vehicle, propranolol 5 mg/kg, prazosin 1.5 mg/kg, or [prazosin 1.5 mg/kg + propranolol 5 mg/kg]), one-way ANOVA within this single day indicated a near-significant main effect of drug treatment, $F(3, 28) = 2.86$, $p = 0.055$. When the prazosin dose was further increased to 2.0 mg/kg on day 5, one-way ANOVA within this single day revealed a significant effect of treatment, $F(3, 29) = 5.35$, $p < 0.01$; [prazosin 2.0 mg/kg + propranolol 5 mg/kg] suppressed alcohol intake relative to vehicle ($p < 0.01$), propranolol 5 mg/kg ($p < 0.05$), or prazosin 2.0 mg/kg treatment ($p < 0.05$). There were no significant differences in water intake among the treatment groups on days 1–3, 4 or 5 (data not shown).

At the start of access on day 5 all rats with the exception of two in the [prazosin 2 mg/kg + propranolol 5 mg/kg] group investigated the alcohol and water tubes within 1 minute of

placement on the cage (of the two that did not, one was active but ignored the bottles for > 1 minute and the other initially remained at the rear of the cage before actively investigating the bottles at just after 1 minute). At completion of the 2-hour alcohol access, all rats chewed on a chow pellet within 15 seconds after it was slipped into the cage.

STUDY 2

After the third experiment in Study 1, the rats received no further alcohol for 3 weeks before a final test of propranolol and/or prazosin effects on alcohol intake, with results shown in Fig. 5. Scheduled alcohol access was available for 2 hours on days 1, 2 and 4, and for only 1 hour on day 5. During the previous experiments most alcohol drinking was observed to occur in the first 15–30 minutes of each 2-h access, so alcohol intakes in this experiment were evaluated across all alcohol access days, even though day 5 access was limited to 1 hour. There was a significant treatment × day interaction in effects on alcohol intake, $F(9, 83) = 3.97$, $p < 0.001$. In the vehicle-treated group there were no differences in alcohol intake among days, confirming that most alcohol drinking occurred in the first hour of access and justifying repeated measures analysis over all 4 days of alcohol access sessions. Treatment with propranolol 5 mg/kg did not significantly alter alcohol intake relative to vehicle treatment in any daily session; prazosin 2 mg/kg suppressed alcohol intake only on the first day of treatment ($p < 0.001$). In contrast, [prazosin 2 mg/kg + propranolol 5 mg/kg] suppressed alcohol intake on all alcohol access days ($p < 0.001$ on day 1, $p < 0.01$ on days 2, 4 and 5). There were no significant differences in alcohol intake among the treatment groups on the pre-drug day or on post-drug day 3. There were no significant differences in water intake (data not shown) among the treatment groups on the pre-treatment day or on post-drug day 3. It was not possible to achieve normal distribution and equal variance of water intake data during the four drug treatment days in which intakes were determined. Analysis of water intakes on individual drug treatment days with Kruskal-Wallis one-way ANOVA on ranks did not identify significant changes among the treatments on any day. However, when average water intakes across all 4 days (0.6 ± 0.1, 0.4 ± 0.1, 1.9 ± 0.5, 1.4 ± 0.1 g/kg for vehicle, propranolol, prazosin, or [prazosin + propranolol] treatment, respectively) were analyzed by one-way ANOVA there was a significant treatment effect, $F(3, 31) = 4.00$, $p < 0.05$; prazosin treatment increased water intake compared to either vehicle or propranolol ($p < 0.05$ for each).

With regard to potential drug-induced sedation or motor impairment, on day 3 (in the absence of alcohol), 1 ml of the sucrose + saccharin solution presented to each rat when each alcohol access session normally started was consumed within 1 minute by every rat in every treatment group. When this test was repeated at the midpoint of the 2 hour access period, the sweet solution was again consumed within 1 minute by every rat in every treatment group. Locomotor activity (number of infra-red beam breaks/5 minutes) in the interval between the two presentations of sweet solution was 461±21, 591±31, 411±31 and 395 ±28 for the rats receiving vehicle, propranolol, prazosin or [prazosin+propranolol], respectively. There was a significant drug effect on locomotor activity, $F(3, 29) = 8.98$, $p < 0.001$; rats receiving propranolol were more active than rats that had received vehicle, prazosin, or [prazosin + propranolol] ($p < 0.01$ for each), which were not significantly different from each other. Day 4 constituted a test of the combined effect of [drug + alcohol] on ingestive behavior. When 1

ml of the sweet solution was presented to each rat at the midpoint of the 2-hour alcohol access period, every rat in every treatment group drank all of the solution within 1 minute.

The effects of drug treatment on BAC on day 5, and the correlations between BAC and preceding 1-hour alcohol intake, are summarized in Table 1. BAC was significantly different among treatment groups, $F(3, 29) = 4.76$, $p < 0.01$, as was alcohol intake (day 5 of Study 2 repeated measures ANOVA; Fig. 4). Combined [prazosin + propranolol] treatment suppressed alcohol intake ($p<0.01$) and BAC ($p<0.05$) relative to vehicle or propranolol treatments. BAC was positively correlated with preceding 1-hour alcohol intake across all treatment groups, $r = 0.85$, $p <0.001$, as well as within each treatment group (Table 1).

DISCUSSION

The selective β-adrenergic receptor antagonist, propranolol, did not produce stable or consistent effects on voluntary alcohol drinking during early withdrawal in alcohol-dependent male P rats given a free-choice between alcohol and water for 2 hours daily. In contrast, combining a low ineffective dose (1 mg/kg) of the α1-adrenergic receptor antagonist, prazosin, with a low ineffective dose of propranolol (10 mg/kg) suppressed voluntary alcohol intake in alcohol-dependent rats during early withdrawal. Gradually increasing the prazosin dose to 1.5 and then 2.0 mg/kg on subsequent consecutive days continued to suppress alcohol intake; this dosage paradigm is comparable to the gradual escalation of prazosin dosage that is used clinically to avoid possible orthostatic syncope (Simpson et al., 2009; Raskind et al., 2003).

After 3 weeks of imposed abstinence, propranolol alone (5 mg/kg) had no significant effect on alcohol intake in rats with a history of alcohol dependence. Prazosin alone (2 mg/kg) suppressed alcohol intake following this imposed abstinence, but only on the first day of treatment. In contrast, these two largely ineffective doses of prazosin and propranolol, when combined, suppressed alcohol drinking on all treatment days

We evaluated the effects of prazosin and propranolol, alone and together, on alertness, locomotor function and ingestive behavior and found that, regardless of drug treatment, the rats: 1) readily investigated the alcohol and water tubes within 1 minute of insertion into the cage; 2) promptly (in <15 seconds) retrieved and chewed a chow pellet inserted into the cage at the end of the alcohol access session; 3) rapidly (in < 1 minute) consumed 1 ml of a sweet solution at time points corresponding to the start and midpoint of the 2-hour alcohol access period; 4) rapidly consumed 1 ml of the sweet solution after 1 hour of alcohol access; and 5) did not exhibit suppressed locomotor activity. Furthermore, water intake was not suppressed by prazosin, propranolol, or prazosin + propranolol in any experiment, and suppression of alcohol intake reported as g/kg BW was accompanied by suppression of alcohol preference based on the alcohol to water drinking ratio. These results illustrate that drug effects on alcohol intake were not mediated by compromised motor function, compromised ingestive behavior, or malaise.

BACs within each of the treatment groups correlated with alcohol intake during the preceding 1 hour. This suggests that the lower blood alcohol levels in the drug-treated groups were secondary to the drug-induced reduction of alcohol intake.

Propranolol in a dose of 10 mg/kg suppressed alcohol intake in Experiment 1a, had no effect on alcohol intake in 3 of the 4 trials in Experiment 1b, and increased alcohol intake in one trial in Experiment 1b. Although similar to the irregular responses to propranolol that we have previously seen in male P rats (Rasmussen et al., 2011), this variability differs from the consistent hypersensitivity to suppressive effects of propranolol seen during operant alcohol-reinforced responding in rats undergoing acute withdrawal from alcohol vapor inhalation (Gilpin and Koob, 2010). There are several differences between the current study and that of Gilpin and Koob (2010), including: operant responding for alcohol *vs* voluntary drinking, use of selected *vs* unselected rats, method of dependence induction, and duration and pattern of prior alcohol drinking history. Of these factors, the method of dependence induction (free-choice drinking in the current study *vs* forced inhalation of ethanol vapor in the Gilpin et al study) may be especially important, since prolonged daily alcohol inhalation is likely to produce a more severe withdrawal response than does free-choice drinking, and the effect of propranolol on alcohol intake may be dependent upon the severity of the withdrawal response. This possibility is supported by the findings of a study examining the effect of propranolol on cocaine use in humans; propranolol decreased cocaine use and increased treatment retention only in the patients who exhibited the most severe cocaine withdrawal symptoms (Kampman et al., 2001).

It is well-established that prazosin alone decreases alcohol drinking in animals and humans in a variety of conditions (Rasmussen et al., 2009a, 2009b; Walker et al., 2008; Froehlich et al., 2013a; Lê et al., 2011; Simpson et al., 2009). In the current study, the effect of prazosin alone on alcohol drinking in rats was inconsistent. This disparity may be due to differences in the presence or absence of alcohol dependence/withdrawal, intensity or duration of dependence or withdrawal when present, or pattern and duration of drug treatment. Further work will be needed to resolve this disparity.

In addition to comparing intakes prior to, during and after vehicle and drug treatments within each experiment, re-randomization and counterbalancing of treatment assignments based on proximal baselines within each experiment provided robust analysis. Overall interpretation is further strengthened by intra-investigation replication of the key result, i.e., combining prazosin with propranolol reduces alcohol drinking more effectively than either drug alone. Baseline/vehicle alcohol intakes remained relatively stable over time and experiments, but the necessarily limited number of subjects in this investigation of P rats that developed alcohol dependence following prolonged voluntary alcohol drinking does not allow further rigorous analysis of potential impact of repeated treatments, and order of treatments, on the stability of alcohol intake in individual rats.

The mechanism by which a combination of prazosin and propranolol reduces alcohol drinking more effectively than either drug alone is not known, but several intriguing possibilities exist. First, noradrenergic activation of α_1- and β-adrenergic receptors can produce different, even opposite, physiological responses (Cooper et al., 2003; Westfall and

Westfall, 2006). This may not be surprising since post-synaptic α_1- and β-adrenergic receptors operate through different signal transduction mechanisms – adenylate cyclase for the β_1-adrenoceptors and calcium mobilization for the α_1-adrenoceptors (Cooper et al., 2003). Activation of one or the other signal transduction mechanism may have a different physiological effect than does activation of both. In addition, pre-synaptic β-adrenoceptors (usually thought to be of the β_2 sub-type) are linked to facilitation of norepinephrine release (Cooper et al., 2003), so a non-subtype selective β-adrenergic receptor antagonist such as propranolol may in some cases also inhibit pre-synaptic norepinephrine release. Thus, blockade of α_1- and/or β-adrenergic receptors as a result of systemic administration of the prototypical α_1- and/or β-adrenergic receptor antagonists (prazosin and propranolol) may be expected to result in different neurochemical activity in brain sites that mediate alcohol drinking when administered separately *vs* together.

Combining well-tolerated drugs such as prazosin and propranolol offers many potential advantages. The current results suggest that combined treatment with prazosin and propranolol may provide greater effectiveness than can either drug alone. Combining prazosin and propranolol also may counteract a negative side effect of one of the drugs. For example, although long-term prazosin treatment of post-traumatic stress disorder (PTSD) has been reported to be associated with sustained loss of the desire to drink alcohol (Raskind and Simpson, 2012), prazosin treatment also can produce reflex tachycardia; addition of propranolol to prazosin in the long-term treatment of PTSD reduced this reflex tachycardia and, as suggested by the current results, may also have contributed to the observed long-term abstinence from alcohol drinking in these patients (Raskind and Simpson, 2012). The combination of prazosin and propranolol may also provide effective treatment for a subset of patients who are not responsive to either drug alone.

In rats with a history of alcohol dependence produced by prolonged voluntary alcohol drinking, combining prazosin + propranolol to decrease noradrenergic signaling mediated by α1- as well as β-adrenergic receptors suppressed alcohol drinking during alcohol withdrawal as well as during prolonged imposed abstinence more effectively than blocking either receptor type alone. These results demonstrate that combination treatment with both prazosin and propranolol, or other CNS-active antagonists of both α_1- and β-adrenergic receptors, may provide especially effective prevention of alcohol relapse for some individuals. Prazosin and propranolol are each well-characterized and FDA-approved, and each has been in clinical use for many years (Westfall and Westfall, 2006). Each has been used chronically by millions of patients and each has long safety and clinical compliance records without significant adverse side effects. Each is orally active and inexpensive. Consequently, these two well-characterized drugs offer great promise for potential use as an effective new approach for treating alcohol abuse and alcohol relapse.

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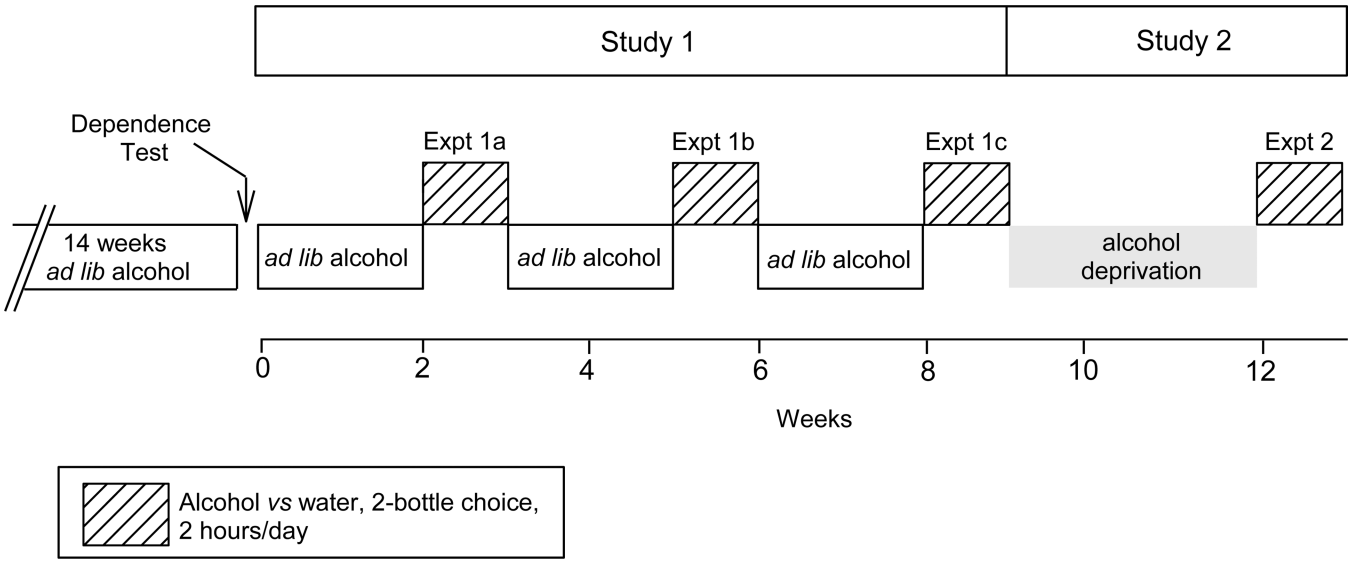

Fig. 1.

Timeline of sequential studies. During *ad lib alcohol* periods, water and alcohol (20%, v/v) were each available 24 hours/day.

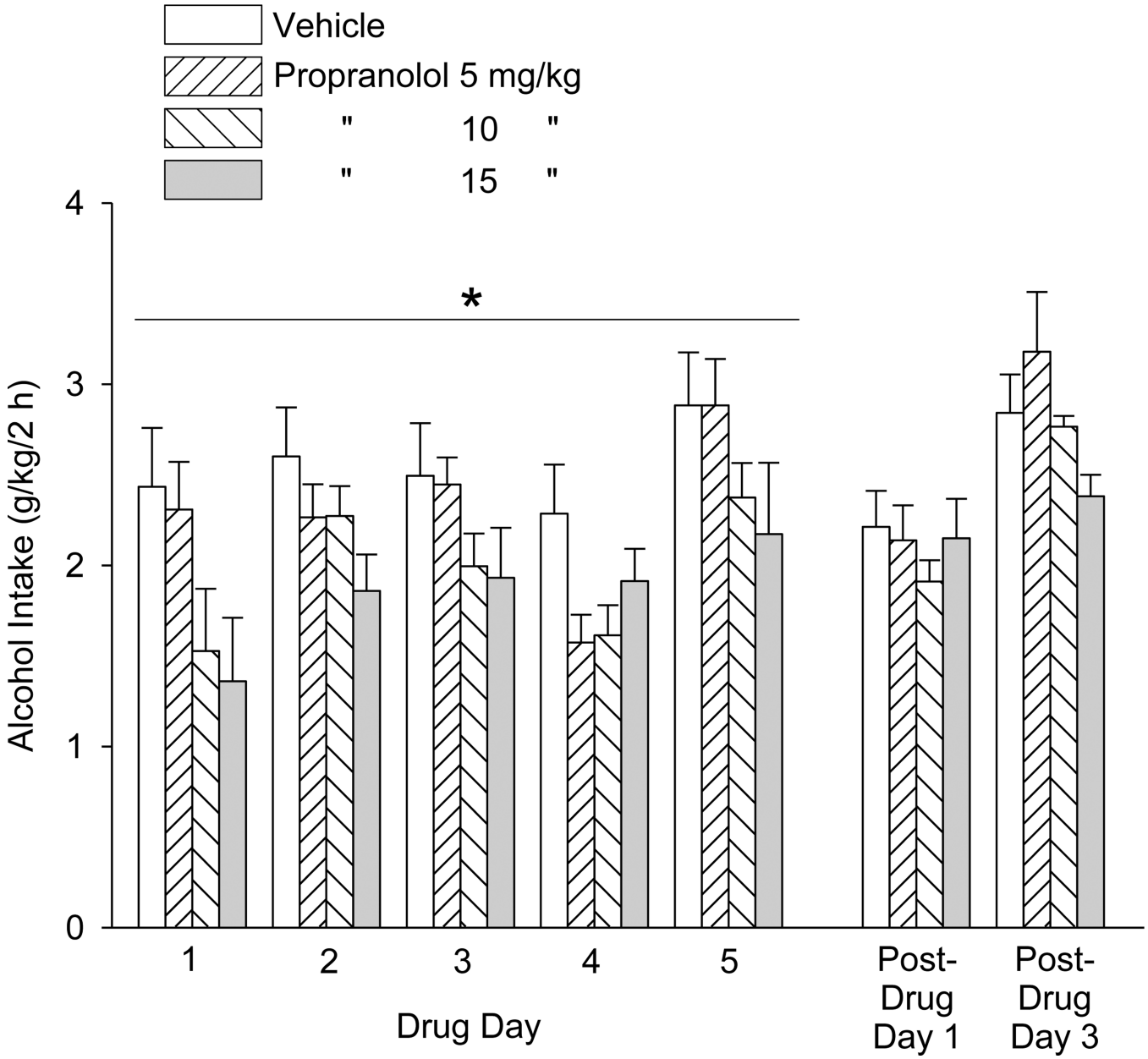

Fig. 2.

Propranolol (5 to 15 mg/kg, IP) effects on alcohol intake (mean ± SEM) during the first 5 days of withdrawal from 24 hour/day alcohol access in alcohol-dependent P rats following long-term voluntary alcohol drinking and development of alcohol dependence. Each bar represents data from 9–10 rats. * $p < 0.05$, 10 or 15 mg/kg *vs* vehicle control treatment, independent of day.

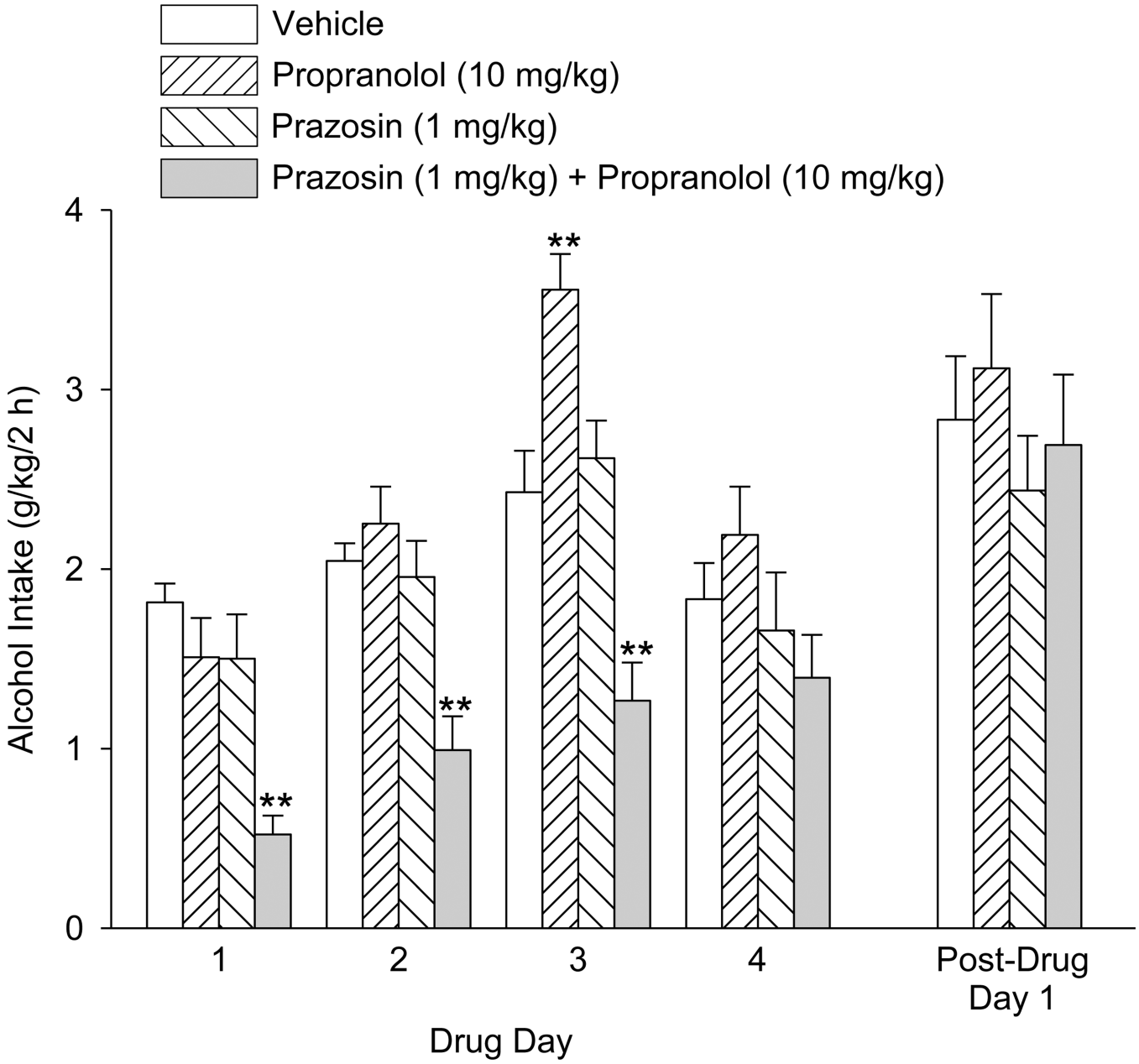

Fig. 3.

Effects of propranolol (10 mg/kg, IP) and/or prazosin (1 mg/kg, IP) on alcohol intake during the first 4 days of withdrawal from 24 hour/day alcohol access following long-term voluntary alcohol drinking and development of alcohol dependence. Each bar represents data from 9–10 rats. ** $p < 0.01$ *vs* vehicle control.

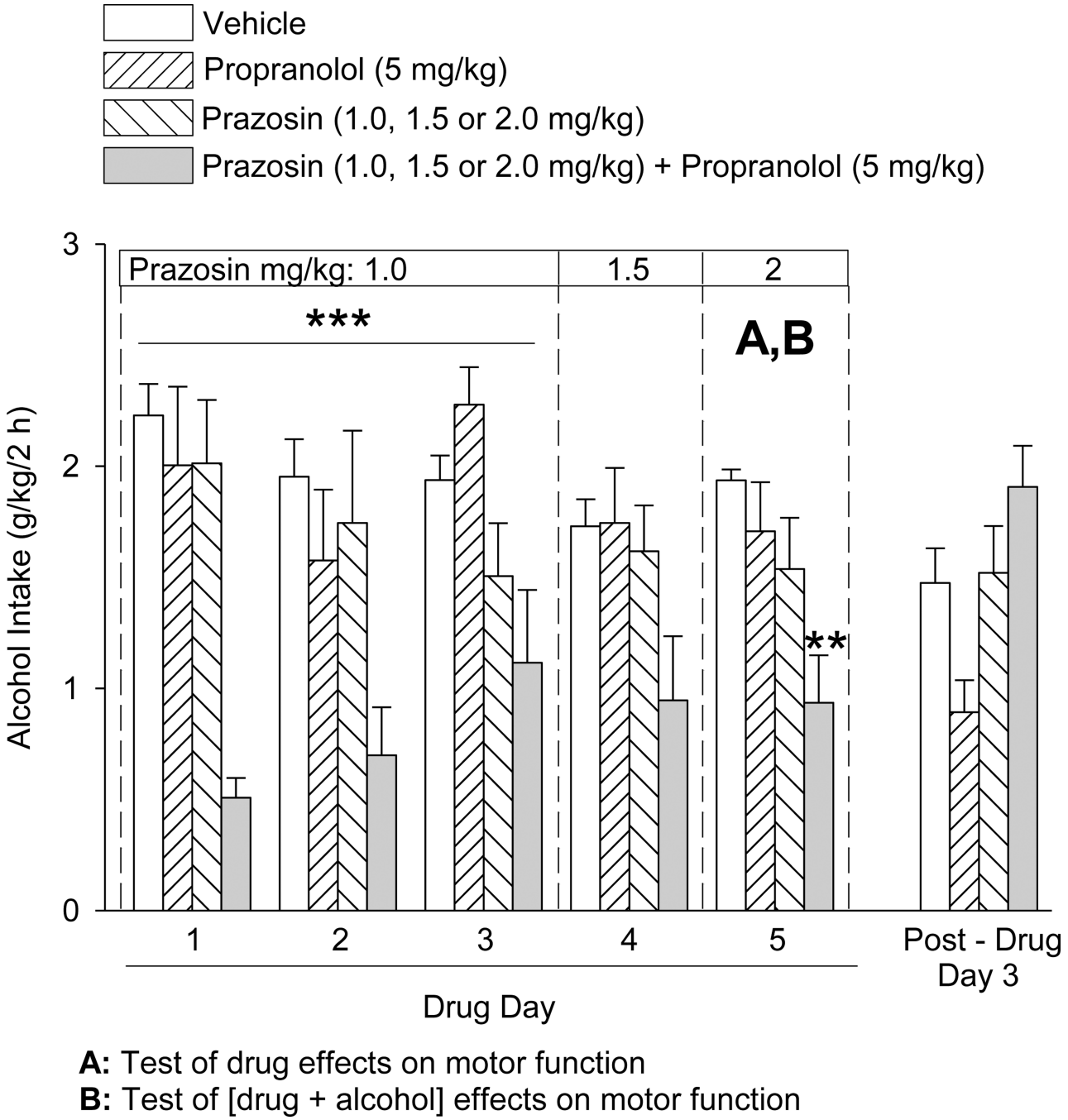

Fig. 4.

Effects of a lower dose of propranolol (5 mg/kg, IP) and/or sequentially increasing doses of prazosin (1 to 2 mg/kg, IP) on alcohol intake during the first 5 days of withdrawal from 24 hour/day alcohol access following long-term voluntary alcohol drinking and development of alcohol dependence. Each bar represents data from 9–10 rats. *** $p < 0.001$ *vs* vehicle control, independent of day; ** $p < 0.01$ *vs* vehicle control.

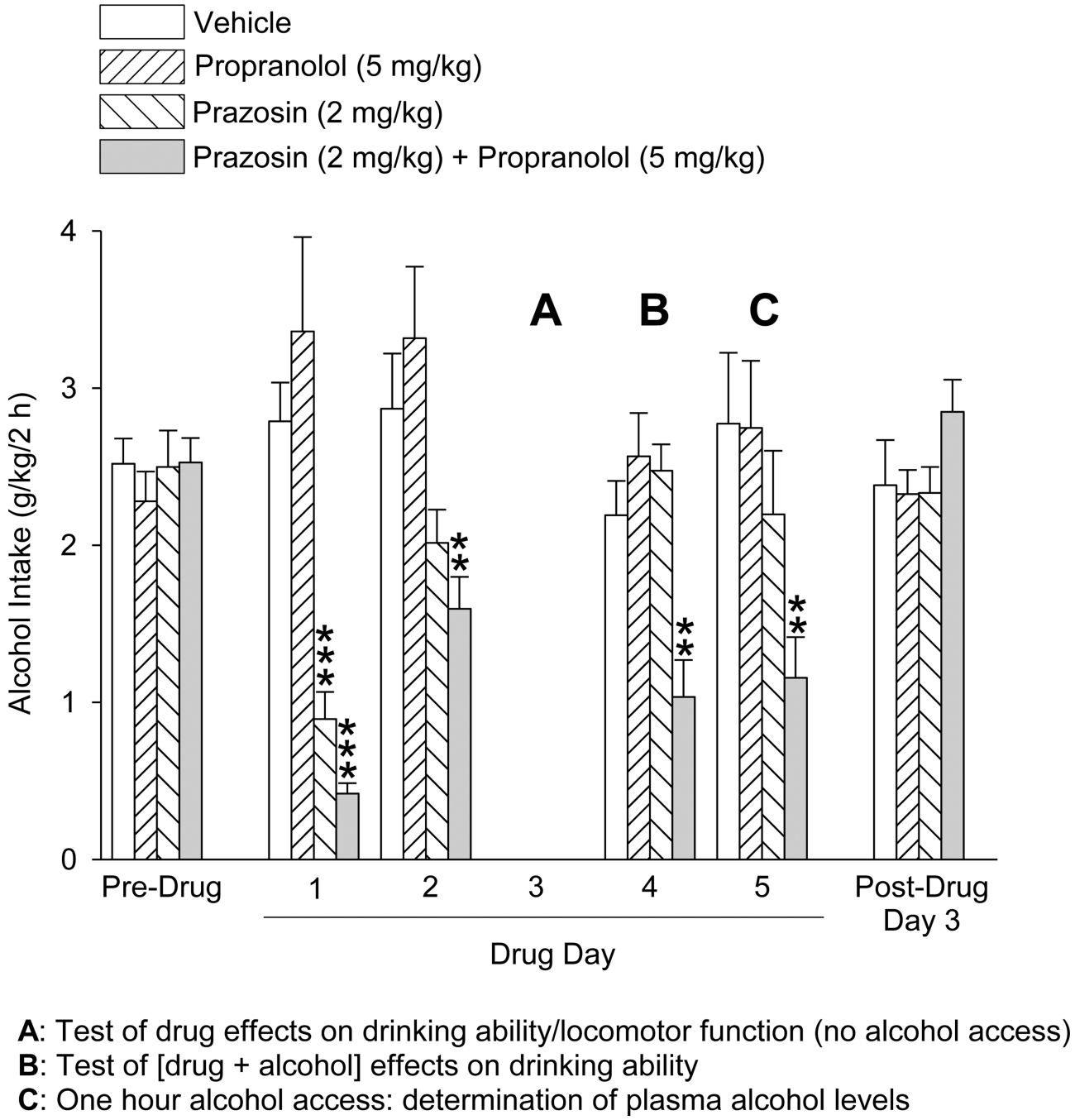

Fig. 5.

Effects of propranolol (5 mg/kg, IP) and/or prazosin (2 mg/kg, IP) on alcohol intake during prolonged imposed abstinence following long-term voluntary alcohol drinking and development of alcohol dependence. Each bar represents data from 9–10 rats. *** $p < 0.001$ *vs* vehicle control; ** $p < 0.01$ *vs* vehicle control.

Table 1

Correlations between alcohol intake (1 hour) and blood alcohol concentration (BAL)

	Alcohol Intake (g/kg/1 hour)	BAC (% w/v)	Correlation coefficient, intake *vs* BAC (*r*)	Correlation significance, intake *vs* BAC (*p*)
Vehicle	2.77±0.45	0.076±0.015	0.88	<0.01
Propranolol (5 mg/kg)	2.75±0.42	0.079±0.020	0.80	<0.05
Prazosin (2 mg/kg)	2.20±0.40	0.039±0.012	0.83	<0.05
Prazosin + Propranolol (2 mg/kg, 5 mg/kg)	1.16±0.26 **	0.016±0.006*	0.74	<0.05

Data are presented as mean ± SEM.

** p<0.01 *vs* Vehicle, Propranolol or Prazosin treatment

* p<0.05 *vs* Vehicle or Propranolol treatment